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Genome Sequence of the Arctic Methanotroph *Methylobacter tundripaludum* SV96▽

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***Methylobacter tundripaludum* SV96^T (ATCC BAA-1195) is a psychrotolerant aerobic methane-oxidizing gammaproteobacterium (*Methylococcales*, *Methylococcaceae*) living in High Arctic wetland soil. The strain was isolated from soil harvested in July 1996 close to the settlement Ny-Ålesund, Svalbard, Norway (78°56′N, 11°53′E), and described as a novel species in 2006. The genome includes *pmo* and *pxm* operons encoding copper membrane monooxygenases (Cu-MMOs), genes required for nitrogen fixation, and the *nirS* gene implicated in dissimilatory nitrite reduction to NO but no identifiable inventory for further processing of nitrogen oxides. These genome data provide the basis to investigate *M. tundripaludum* SV96, identified as a major player in the biogeochemistry of Arctic environments.**

Arctic permafrost environments are methane (CH_4) reservoirs. Ongoing and future changes in the Arctic may contribute to increased turnover of organic carbon, resulting in higher CH_4 emissions. As the sole biological methane filter, methane-oxidizing bacteria (methanotrophs) are important controllers in this process. While both alpha- and gammaproteobacterial methanotrophs are present in circumpolar permafrost regions, including Canada, Siberia, and Svalbard, a dominance of the gammaproteobacterial genus *Methylobacter* was observed (8, 10, 14). *Methylobacter tundripaludum* SV96 was identified as an active methanotroph in its *locus typicus* by RNA-SIP (stable isotope probing) with ^{13}C methane (5, 15).

The draft genome of *M. tundripaludum* SV96 was generated at the DOE Joint Genome Institute (JGI) using a combination of Illumina (1) and 454 technologies (9). The Illumina GAii shotgun library produced 41,446,074 reads (3,149.9 Mbp), a 454 Titanium standard library yielded 500,724 reads, and a paired-end 454 library (7-kb average insert size) delivered 271,299 reads. The 220.6 Mbp of 454 data were assembled with Newbler (version 2.3). Illumina data were assembled with VELVET, version 1.0.13 (16). Reads were assembled after computational shredding using parallel phrap (SPS version 4.24; High Performance Software, LLC). Consed software (2, 3, 4) was used for finishing. Potential base errors and consensus quality were corrected using Illumina data and Polisher software (A. Lapidus, unpublished data). Possible misassemblies were corrected using gapResolution (C. Han, unpublished data), Dupfinisher (6), or sequencing of subcloned bridging PCR fragments. Gaps between contigs were closed by PCR or bubble PCR primer walks using Consed (J.-F. Cheng, unpublished data). Gaps were closed with 1,095 PCRs, yielding a sequence of 3 contigs (2,936,421, 367,513, and 1,544,395 bp). The final assembly represents 190.8 Mbp of 454 data and 2,930.7 Mbp of Illumina data. Sequence annotation and comparative genome analysis are under way with assistance from the MicroScope platform at Genoscope (13).

The *M. tundripaludum* SV96 genome contains one operon for particulate methane monooxygenase (*pmoCAB*) and the recently discovered *pxm* operon (*pxmABC*), encoding a copper membrane monooxygenase of unknown function (12). Genes involved in methanol oxidation (*mxaFI*), H_4folate (*mtdA-fch*) and H4MTP-linked (*fae-mtdB-mch-fhcABCD*) C_1 transfer pathways, membrane-associated quinoprotein formaldehyde dehydrogenase (*ald*), and formate oxidation (*fdsGBACD*) were identified. Genes for a complete RuMP pathway for carbon

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assimilation and the oxidative TCA cycle were present, while the serine cycle was incomplete.

Genes encoding necessary inventory for nitrogen fixation, nitrate transport (*narK*) and assimilation (*narGHJI*), nitrite assimilation (*nirBD*), and ammonia transport (*amtB*) and assimilation (glutamine synthetase/glutamate synthase but not alanine dehydrogenase) were found. Unique thus far to methanotrophic bacteria, a complete urea cycle was detected, as well as a cytochrome cd_1 NirS nitrite reductase and associated cofactors for reduction of nitrite to nitric oxide. Genes encoding nitric oxide reductases were not found. Genes encoding hydroxylamine reductase and its electron-donating partner were identified, suggesting a hydroxylamine detoxification mechanism similar to that found in *Methylosinus trichosporium* OB3b (11). At least 20 homologs of cold shock-related proteins were detected, including 6 homologs of CspD (7).

Nucleotide sequence accession number. The *Methylobacter tundripaludum* SV96 genome sequence is available in GenBank under accession number AEGW00000000.

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